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(71) Applicant *(for all designated States except US)*: UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION[AT/AT]; Vienna International Centre, Wagramerstrasse 5, P.O. Box 300, A-1400 Vienna (AT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : MANIVEL, Venkatasamy [IN/IN]; B4/93/2, Safdarjung Enclave, New Delhi - 110 029 (IN). RAO, Kanury, Venkattasubba [IN/IN]; N-9B, Saket, New Delhi - 110 017 (IN). PANDA, Subrat, Kumar [IN/IN]; 408, Asiad Village, New Delhi - 110 049 (IN).

(74) Agents: ITZE, Peter et al.; Casati & Itze, Amerlingstr. 8, A-1061 Vienna (AT).

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(57) Abstract

The invention relates to a peptide showing cross-reactivity with anti-hepatitis B surface antigen (HBsAg) antiserum and comprising the "a" epitope of HBsAg.

Peptide CS[124-147] (subtype adw) and its analogs

Peptide	Sequence
CS	CTTPAQGNSMFPSCCCTKPTDGNC
CS ΔA[130]	CTTP_QGNSMFPSCCCTKPTDGNC
CS ΔQ[129]	CTTPA_GNSMFPSCCCTKPTDGNC
CS[129→A]	CTTPAQGΔSMFPSCCCTKPTDGNC
CS[129→G]	CTTPAQGNS(G)MFPSCCCTKPTDGNC
CS[130→A]	CTTPAQGNSMFΔSCCCTKPTDGNC
CS[131→A]	CTTPAQGNSMFPΔCCCTKPTDGNC
CS[132→M]	CTTPAQGNSMFPSCCCT(Met)KPTDGNC
CS[135→A]	CTTPAQGNSMFPSCCCTKΔTDGNC
CS[136→N]	CTTPAQGNSMFPSCCCTKPTNGNC

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PEPTIDE SHOWING CROSS-REACTIVITY WITH THE ANTI-HEPATITIS B SURFACE ANTIGEN ANTISERUM

The invention relates to a peptide showing cross-reactivity with anti-hepatitis B surface antigen (HBsAg) antiserum.

The major protein (or S protein) of the hepatitis B surface antigen (hereinafter referred to as "HBsAg") comprises a length of 226 amino acids and encodes a group-specific determinant, also known as "a" determinant, that is common to all subtypes of the hepatitis B virus (HBV). In addition to the group-specific determinant there are two groups of mutually exclusive subtype-specific determinants, d or y and w or r, resulting in four major subtypes of the virus: adw, adr, ayw and ayr. Several studies have shown that immunization with the major protein alone can protect humans against HBV infection, which indicates that this protein contains epitopes necessary for eliciting protective antibodies. Further observations have shown that immunization with the major protein representing a specific subtype of HBV can protect against infection with all other subtypes. This is an indication that the group-specific or "a" determinant plays an important role in eliciting antibodies that provide protection in cross-reactions. Therefore it was found to be important that a peptide for a vaccine against hepatitis B contains one or more of the "a" epitopes of hepatitis B surface antigen.

While the prior art is replete with studies in respect of immunization against hepatitis in general, all these studies had one factor in common viz. the vaccine comprised of either a weak or a dead hepatitis B surface antigen. Non of the prior art even remotely suggested employing a synthetic "antigen" which was capable of emulating the natural antigen.

Accordingly, it is the primary object of the present invention to provide a peptide for use in the manufacture of vaccine against various infections.

More particularly, it is the object of the present invention to provide a peptide for use in the manufacture of vaccine against hepatitis B.

It is yet an object of the present invention to manufacture a peptide which can emulate the epitope of a naturally occurring hepatitis B surface antigen.

It is a further object of the present invention to provide a peptide which shows a cross-reactivity with antihepatitis B surface antigen (HBsAg) antiserum.

These objects of the present invention are achieved by providing a peptide for vaccine against hepatitis B containing one or more "a" epitopes of hepatitis B surface antigens.

While attempts were made to localize the "a" determinant of HBsAg it was noticed that this determinant does not represent a linear sequence of the protein. Rather, it seems that it concerns a conformational epitope, which is formed by extensive intra- and intermolecular disulfide bonds. However, it was possible t localize two of these epitopes on synthetic peptides. They represent the amino acid residues of the sequence 122 - 137 and 139 - 147. A peptide was synthesized representing the sequence area 124 - 147 of HBsAg of the subtype adw (Fig. 1). The precise sequence of this peptide is shown in Fig. 1 in form of the one letter code. The sequence contains five cysteine residues, whereby one each is situated at the amino- and carboxy-terminii and a series of three consecutive cysteine residues is situated at the positions 137, 138 and 139. After the cleavage of the protected peptide from the carrier resin and the following work-up, it was observed that the peptide spontaneously oligomerized with the formation of disulfide bonds yielding a heterogenous mixture of multiple forms with molecular weights ranging from 8 - 35 kDa. This is proved by Fig. 2. This peptide will hereinafter be referred to as OS[124-147]. The peptide OS[124-147] shows a high degree of cross-reactivity with a polyclonal anti-HBsAg antiserum while neither partially reduced trimeric forms of

the peptide nor completely reduced monomer forms of the peptide showed any cross-reactivity with the anti-HBsAg antiserum. This is disclosed by the diagram as shown in Fig. 3 in which the trimeric forms are designated by TS[124-147] and the monomer forms by MS[124-147]. This leads to the conclusion that the peptide OS[124-147] contains conformational, disulfide-dependent epitopes that are also present on the native S protein. When using HBsAg subtype-specific antisera it was observed that the HBsAg-related epitopes that are formed by the peptide OS[124-147] represent the group-specific or "a" epitopes of HBsAg.

Thus, the present invention in essence concerns a peptide which is capable of mimicking the hepatitis B surface antigen so that it can generate antibodies against hepatitis in animals in the same way as the hepatitis B surface antigen.

A method for the manufacture of a peptide according to the invention for use in the formation of vaccines comprises building in any conventional manner, on a conventional support, a predeterminated sequence of amino acids such as herein described, said predetermined sequence corresponding to that of hepatitis B surface antigen epitope, coupling the amino terminus of the peptide so formed with a fatty acid, subjecting said peptide to cleavage to separate the peptide from said support and simultaneously deprotecting the amino acid side chains of the peptide by treating it with an acid of the kind such as herein described in the presence of a scavenger, washing the product so formed with a solvent, extracting said product and lyophilizing said product to yield a crude peptide and purifying the crude product in any appropriate manner to obtain said peptide for use in the manufacture of vaccine.

The supports on which the predetermined amino acid sequence is built are commercially available. The most commonly used supports are solid resins. The applicants have obtained excellent results by employing 4-methylbenzhydrylamine resin as solid support. The building of individual amino acid on the support is well known in the art. Such coupling may be carried out in the presence of any

conventional activator, carbodiimide being the most preferred. Again, while depending upon the vaccine, any amino acid sequence may be built for vaccine against hepatitis, the following twelve animo acids viz. cystein proline alanine, glutamine, glycine, asparagine, serine, methionine, phenylalamine, lysine and aspartic acid were employed.

While the applicants are not aware of any teaching which discloses coupling of amino terminus of the peptide with any fatty acid, they have discovered that such coupling plays an important role in the efficacy of the final vaccine. Any fatty acids may be employed for the purpose. Such coupling completes the basic peptide structure that is required and thereafter, it is no longer necessary to retain the support. The simultaneous cleavage of the peptide from the solid support and deprotection of amino acids side chains are achieved extremely well by employing hydrogen fluoride in the presence of thioanisole, especially 10 % thioanisole, as a scavenger. Such acid treatment clips unwanted groups from the peptide. Common acids that may be employed are hydrochloric acid, trifluoroacetic acid, fluoromethano sulphonic acid etc. Ethanedithol has also shown good results as a scavenger. The cleaved peptide is, thereafter, washed with conventional washing liquids, for instance ethyl acetate or dry ether, and extracted with 1M acetic acid of other appropriate solvents. The number of washing cycles would depend upon the type of the solvent employed.

For purification purposes, the crude product is dissolved in deionized water. This solution is then dialyzed thoroughly against deionized water over a period of 24 hours with the dialyzing medium being changed at least five times and a membrane having a molecular weight cut-off of 1.200 daltons is used. The dialyzed solution is frozen and lyophilized to provide a quantitative yield of the product.

In order to obtain an efficient candidate vaccine, the peptide OS[124-147] must be immunogenic, i.e., it must be capable of eliciting an antibody response in the absence of a carrier protein. To prove this, Balb/c mice and New Zealand white

rabbits were immunized with the peptide OS[124-147] using the complete and incomplete Freud's adjuvant system. Anti-peptide responses were obtained in both hosts indicating that the peptide OS[124-147] is indeed immunogenic. Furthermore, both the mice and the rabbits showed antisera that cross-reacts equally well with this peptide or the native HBsAg (see Fig. 4), implying that the peptide OS[124-147] represents a substantially accurate replica of the native epitope. The rabbit anti-peptide antiserum showed cross-reactions with a variety of HBsAg subtypes (see Table 2), which indicates that at least a significant proportion of the antibodies are directed against the "a" epitope. Finally it was observed that rabbit anti-OS[124-147] antisera can also immunoprecipitate infectious hepatitis B virus particles (Dane particles) in vitro (Fig. 5).

To identify the amino acid sequences that form the HBsAg-related "a" epitope, nine analogs of peptide OS[124-147] were generated. These analogs were partly obtained either by substitution, deletion or chemical modification of an amino acid in the sequence (see Fig. 6). Using antisera specific for HBsAg of the subtype ay as a probe for the "a" epitope on peptide OS[124-147] (subtype adw), it was found that the chemical modification of either methionine 133 or lysine 141 decreased antibody binding capacity by at least 80% (Fig. 7). This proves that both methionine 133 and lyine 141 represent individual constituents of the epitope which represent the "a" epitope of HBsAg. The methionine 133 - lysine 141 dependent "a" epitope has not been found yet.

To ascertain the dominance of the "a" epitope in its natural environment, human sera from fifty individuals who had been vaccinated against hepatitis B were used. The sera were tested for reactivity with each of the analogs of peptide OS[124-147], as shown in Fig. 6. The results are shown in Fig. 8 and the conclusions drawn therefrom are that all of these fifty sera were capable of recognizing the peptide OS[124-147] and that the "a" epitopes comprising methionine 133 and lysine 141 represent the dominant recognition site for 96 % (48 of 50 sera)

of the serum samples. All told, it was recognized that methionine 133 and lysine 141 is a dominant constituent of the epitope which is present in the HBsAg of humans.

The results as described above show that the peptide OS[124-147] represents a group-specific determinant of HBsAg. This "a" epitope is a dominant epitope and was recognized by all fifty of the human HBsAg sera tested. Having shown that the peptide OS[124-147] is immunogenic in both mice and rabbits with Freud's adjuvant system, the system cannot be used in humans, because of the toxicity of Freud's adjuvant system in humans. To find a vaccine it is therefore necessary that the immunogenicity of the peptide OS[124-147] is demonstrated in alum, because alum is the only adjuvant approved for human use. To achieve the requisite antibody response a myristic acid residue was attached to the amino-terminus of the peptide so as to achieve a higher aggregation via micelle-like interactions. The myristylated peptide (OS[124-147]) showed a quantitative aggregation, which was determined by sucrose density gradient centrifugation. In addition, the myristylated peptide showed immunogenic reactions in separate strains of mice and in rabbits (see Table 3).

To draw conclusions on humans, the system was extended to primates (rhesus monkeys) in order to test a system that was closer to humans. Four monkeys were tested in this experiment. One was immunized with a commercially available hepatitis B vaccine at the dose usual for humans (20 μg). The remaining three monkeys were immunized with either 50 μg, 250 μg or 500 μg of the myristylated peptide OS[124-147] that was adsorbed onto alum. Respective antibody responses were found, whereby the primary immunization was followed by a booster immunization a month later (see Table 4). This alum adsorbed myristylated peptide OS[124-147] is highly immunogenic in rhesus monkeys at all doses tested. At a 50 μg dose the obtained anti-peptide titer was comparable to that obtained in the monkey vaccinated in the usual manner. On the other hand, the monkey immunized with a 250 μg dose showed a twice higher antibody response. Immunization with a 500 μg dose of peptide showed a thirteen-fold higher antibody reaction as opposed to monkeys

vaccinated in the usual manner. In all of the four monkeys at least 80 % of the anti-OS[124-147] response was directed against the methionine 133 - lysine 141 dependent "a" epitope. Thus immunization with a comparable dose of either the common vaccine (20 μg) or myristylated peptide OS[124-147] results in substantially identical levels of antibodies directed against the methionine 133 - lysine 141 dependent "a" epitope, whereas a higher dose of peptide can be used to obtain a significantly higher antibody response. The latter can be used to reduce the number of doses required to achieve the respective protection against hepatitis B.

To obtain an expression of the "a" epitope that is several times higher it is possible to substitute the the amino acid asparagine at position 144 for aspartic acid. Furthermore, it was observed that although the majority of antibodies elicited in response to peptide OS[124-147] is directed against the "a" epitope, a minor proportion of antibodies were also directed against the subtype "d" epitope which is resident in the amino-terminal portion of the peptide. The peptide OS[124-147] was derived from HBsAg of subtype adw.

To achieve the optimization of the immunogen, two peptides were synthesized. One peptide represented the 124-147 sequence of HBsAg of subtype adw, but with aspartic acid at position 144. Then there was the other peptide that represented the sequence 124-147 of HBsAg of subtype ayr. Both protected peptides, which were grafted onto a resin, were myristylated and mixed in equimolar quantities, whereupon they were cleaved from the resin to obtain a co-oligomer of both peptides. This resulted in a single immunogen that encodes in an optimal manner the "a" epitope and, in addition, both subtype-specific "d" and "y" epitopes contained in the amino-terminal portions, namely as individual component peptides (see Fig. 10). This immunogen (peptide OS[D/N-ayr]) can produce, in addition to the anti-a response, anti-d and anti-y responses, which would result in a wide spectrum of anti-HBsAg responses that are indifferent to subtype and which bring about improved efficiency in neutralizing HBV infections.

Preparation method

A solid phase peptide synthesis of the sequence between residues 124 and 147 of hepatitis B virus surface antigen (subtype adw) is carried out, whereby 4-methylbenzhydrylamine resin is used as solid support. The individual coupling reactions are carried out in the presence of a four-fold excess of protected amino acids using carbodiimide as activator. Usually, the synthesis is performed in a 0.5 mmole scale using 2 mmoles of each protected amino acid. Twelve protected amino acids are used in the synthesis, namely cysteine, threonine, proline, alanine, glutamine, glycine, asparagine, serine, methionine, phenylalanine, lysine and aspartic acid. After the peptide assembly simultaneous cleavage from the solid support and the deprotection of amino acid side chains is achieved with hydrogen fluoride at a temperature below 0°C using 10% thioanisole as scavenger. After the cleavage of the resin and its subsequent three-times washing with ethyl acetate or dry ether, the cleaved peptide is extracted with 1M acetic acid. The acetic acid extract is then frozen and lyophilized to yield the crude product, which is dissolved in deionized water. This solution is then dialyzed thoroughly against deionized water over a period of 24 hours with the dialyzing medium being changed at least five times and a membrane having a molecular weight cut-off of 1200 daltons is used. The dialyzed solution is frozen and lyophilized to provide the product.

The obtained product is subjected to immunological tests and evaluation to ascertain that an accurate mimic of the "a" determinant has indeed been formed. Electron microscopic examination of an aqueous solution of this product can also be carried out to verify the presence of hepatitis B surface antigen-like particles.

Table 1 shows that antisera that are specific for three distinct subtypes of hepatitis B surface antigen are capable of recognizing the oligomeric peptide OS[124-147] to a large extent. However, neither the partially reduced trimetric form

(TS[124-147]) nor the completely reduced monomeric form (MS[124-147]) are capable of recognizing this peptide. This clearly shows that the hepatitis B surface antigen-related epitope is expressed by the peptide OS[124-147] and represents the group-specific "a" determinant and that the antigenicity is dependent on the disulfide bonds.

HBsAg subtype-specific antisera (WHO International Reference Standard) were screened for reactivity against the indicated peptides at a dilution of 1:100. A P/N value in excess of 2.0 is regarded as a positive value.

Table 1

Subtype-specifity of antiserum	P/N OS[124-147]	TS[124-147]	MS[124-147]
ad	64.5	3.2	0.3
ay	54.0	5.4	0.5
ar	47.8	4.3	0.6

In order to further explore the potential of the peptide as a hepatitis B vaccine, polyclonal rabbit antisera effective against the peptide OS[124-147] were screened for cross-reactivity with the International Standard hepatitis B surface antigen preparations of six different subtypes.

Table 2 shows the results of this experiment, whereby a parallel set of assays with polyclonal anti-hepatitis B surface antigen antisera were used as positive control. Anti-peptide OS[124-147] antisera reacted to comparable extents with all six of the subtypes tested. The implication of these results is that an immunization with the indicated peptide OS[124-147] will produce antibodies capable of recognizing all strains of the hepatitis B virus. A 1:500 dilution of either rabbit anti-OS[124-147] or horse anti-HBsAg was used for this experiment. The various HBsAg-subtypes used are indicated in Table 2.

TABLE 2

HBsAg subtype	P/N with anti-HBsAg	with anti-OS[124-147]
adw	10.6	6.3
ayw	18.2	4.7
ayr	5.9	4.7
ayw2	8.6	5.4
a2dw	12.2	6.7
adw2	9.9	7.9

Table 3 shows the immunogenicity of myristylated peptide OS[124-147] in alum. The results shown in the table represent the anti-peptide titers obtained after a primary immunization followed by a booster dose given four weeks later. The peptides adsorbed onto alum were used as the immunogen, whereby the dose was either 20 μg per mouse or 200 μg per rabbit.

TABLE 3

Host	Strain	No.	Anti-OS[124-147] (1/titer)
Mice	Balb/c		1300
	C57 B1/6		2500
Rabbits	NZW	1	8270
		2	7970
		3	20800

Table 4 shows the immunogenicity of alum-adsorbed myristylated peptide OS[124-147] in rhesus monkeys. The anti-peptide titers were obtained after a first immunization followed by a booster immunization one month later.

TABLE 4

Monkey No.	Immunogen	Dose (μg)	Anti-OS[124-147] (1/titer)
1757	Vaccine	20	7,200
745	OS[124-147]	50	7,200
1442	OS[124-147]	250	17,000
1109	OS[124-147]	500	95,000

Fig. 1 shows the primary sequence of the peptide OS[124-147] (subtype adw). The single-letter code for amino acids was used here.

Fig. 2 shows a polyacrylamide gel electrophoresis of the peptides OS[124-147] and TS[124-147].

For this assay two micrograms of each peptide were dissolved in an equal volume of mercaptoethanol-deficient sample buffer and resolved on an 18% polyacrylamide gel. Visualization was effected by silver staining. In this Fig. lane 1 shows the molecular weight marker, lane 2 the peptide OS[124-147] and lane 3 the peptide TS[124-147].

Fig. 3 shows the extent to which a polyclonal anti-HBsAg antiserum recognizes the peptide OS[124-147], but not the peptide TS[124-147] or the peptide MS[124-147]. The tests were carried out in an ELISA assay. The data are expressed as P/S ratios, whereby S represents the absorbance obtained with an irrelevant monomere peptide.

Fig. 4 shows the titration profiles of polyclonal anti-OS[124-147] sera versus HBsAg and peptide.

Mouse (A) or rabbit (B) anti-peptide antisera were titrated against either peptide OS[124-147] (o) or native HBsAg(o) in the ELISA assay. Data are expressed as P/N ratios where N indicates the absorbance obtained with a 1:100 dilution of preimmune serum against the respective antigen.

Fig. 5 shows anti-OS[124-147] antibodies precipitated with Dane particles. The purified Dane particles were subjected to immunoprecipitation either with rabbit preimmune serum (column a) or rabbit polyclonal anti-OS[124-147] serum (column b). The HBVDNA contained in the supernatant (top) or the protein A pellet (bottom) was identified by spot hybridization.

Fig. 6 shows the peptide OS[124-147] and its analogs. The positions where individual amino acids were deleted, substituted or modified are underlined.

Fig. 7 shows the identification of the amino acid residues used in an anti-ay binding assay. The peptide OS[124-147] and its analogs were coated separately onto wells of an ELISA plate and the reactivity with guinea pig anti-HBsAg (ay) at a final dilution of 1:200 was determined. The relative antigenicity (RA) represents the ratio of absorbance obtained for a given analog versus that obtained for the parent peptide after the subtraction of the background.

Fig. 8 shows the identification of human anti-HBsAg antibody binding residues on the peptide OS[124-147]. The cumulative results for fifty independent sera are presented here as the percent of the total number of samples which show the reactivity with the analogs relative to the parent peptide OS[124-147].

Fig. 9 shows the sucrose density gradient profile of myristylated peptide OS[124-147].

200 μg of myristylated peptide OS[124-147] in 100 μl was layered onto a 10 - 30% sucrose gradient and centrifuged in an SW 50.1 rotor for 20 hours. At the end of the run the fractions were collected and the percentage of sucrose in each fraction was determined by refractometry. The amount of peptide present in each fraction was determined by the ninhydrin test. Special note must be taken of the fact that all peptides entered the gradient solution, whereby the majority sedimented at the bottom.

Fig. 10 shows the primary sequences of the individual component peptides of the peptide OS[D/N-ayr].

Summarized can be said that a synthetic peptide was found representing the sequence between residues 124-147 of the hepatitis B surface antigen and showing spontaneous oligomerization to form a conformational, disulfide- and oligomer-dependent group-specific or "a" antigenic determinant of the hepatitis B surface antigen. Furthermore, it was shown that the peptide OS[124-147] mimics the corresponding epitope of the native antigen withhigh precision and represents a dominant constituent of the epitope of the hepatitis B surface antigen when introduced into the human immune system. The peptide OS[124-147] is immunogenic in mice as well as rabbits and the antibodies elicited against this peptide are capable of recognizing a plurality of hepatitis B surface antigen subtypes.

Finally, the peptide OS[124-147] forms spherical and tubular aggregates in aqueous solutions which are reminiscent of native hepatitis B surface antigen particles.

Thus, the peptide OS[124-147] represents an antigenic and structural mimetic of hepatitis B surface antigen and consequently it is a candidate vaccine for hepatitis B.

Claims:

1. A peptide showing cross-reactivity with anti-hepatitis B surface antigen (HBsAg) antiserum which comprises the 'a' epitope of HBsAg, characterized in that it is an oligomer of the amino acid sequence

C T T P A Q G N S M F P S C C C T K P T D G N C

or its analogs or homologs preferably with a molecular weight of 8 - 35 kDA.

2. A peptide as claimed in claim 1, characterized in that the analogs or homologs comprise the following amino acid sequences:

Analogs of the peptide OS [124-147] (subtype adw)

Peptide:	Sequence:
OS	C T T P A Q G N S M F P S C C C T K P T D G N C
OS Δ A^{128}	C T T P _ Q G N S M F P S C C C T K P T D G N C
OS Δ Q^{129}	C T T P A _ G N S M F P S C C C T K P T D G N C
OS[N^{131} → A]	C T T P A Q G A S M F P S C C C T K P T D G N C
OS[M^{133} → Ox]	C T T P A Q G N S (O) M F P S C C C T K P T D G N C
OS[P^{135} → A]	C T T P A Q G N S M F A S C C C T K P T D G N C
OS[S^{136} → A]	C T T P A Q G N S M F P A C C C T K P T D G N C
OS[K^{141} → Me]	C T T P A Q G N S M F P S C C C T (Me) K P T D G N C
OS[P^{142} → A]	C T T P A Q G N S M F P S C C C T K A T D G N C
OS[D^{144} → N]	C T T P A Q G N S M F P S C C C T K P T N G N C

3. A peptide as claimed in claim 1 or 2, characterized in that the 'a' epitope is determined by the amino acids methionine at the position 133 and lysine at the position 141.

4. A peptide as claimed in one of the claims 1 to 3, characterized in that the 'a' epitope comprises aspartic acid at position 144 as amino acid.

5. A peptide as claimed in one of the claims 1 to 4, characterized in that the peptide comprises in addition to the 'a' epitope other additional epitopes of HBsAg, in particular the "d" and/or "y" epitope.

6. A vaccine against hepatitis B, characterized in that it contains a peptide as claimed in claims 1 to 5 in combination with alum as adjuvans, whereby a myristic acid recidue is added to the amino-terminus of the peptide.

7. An application of the peptide as claimed in one of the claims 1 to 5 as reagent in the diagnosis of hepatitis B virus infection.

Fig. 1

CTTPAQGNSMFPSCCCTKPTDGNC

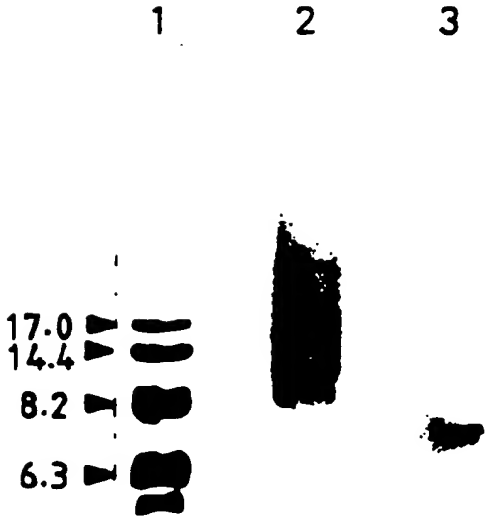


Fig.2

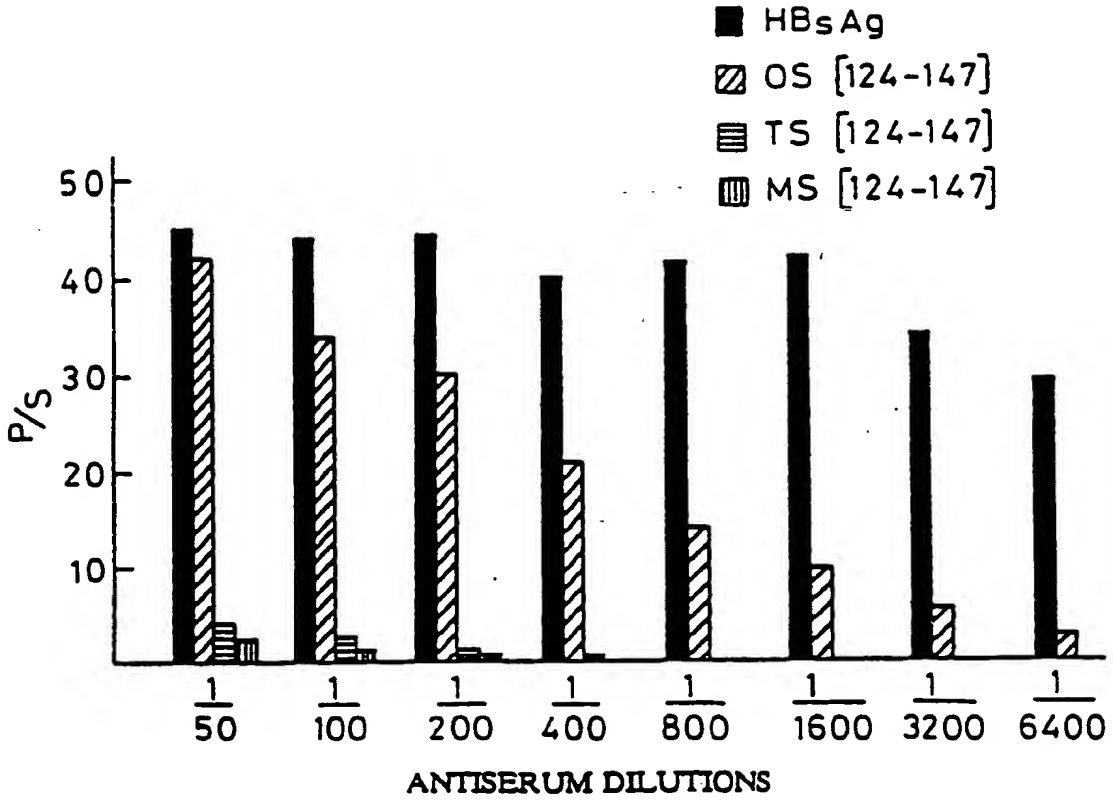
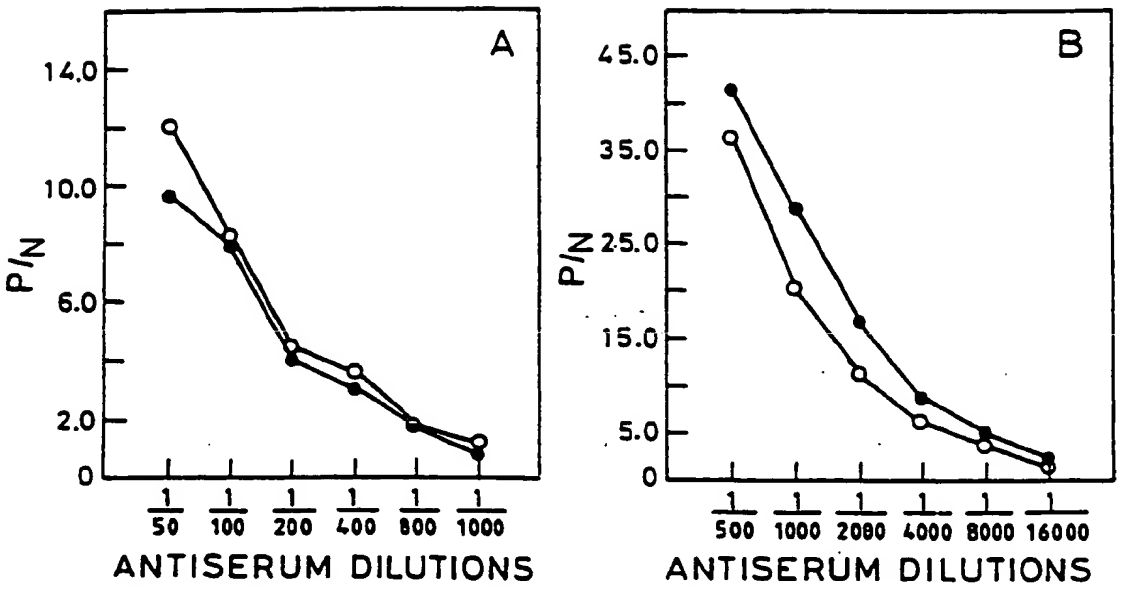

HBsAg
OS [124-147]
TS [124-147]
MS [124-147]
P/S
50
40
30
20
10
1/50
1/100
1/200
1/400
1/800
1/1600
1/3200
1/6400
ANTISERUM DILUTIONS

Fig. 3

Fig. 4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35.0
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P/N
1/500 1/1000 1/2000 1/4000 1/8000 1/16000
ANTISERUM DILUTIONS

Fig. 5

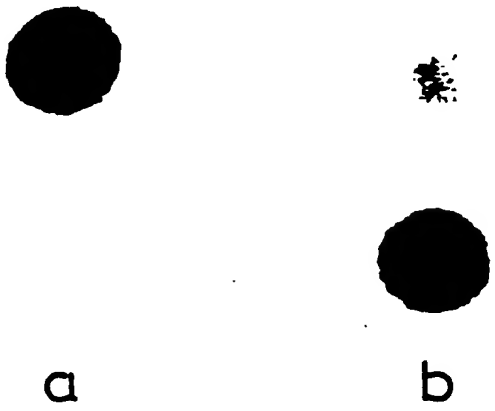
a
b

Fig.6

Peptide OS[124-147] (subtype adw) and its analogs

Peptide	Sequence
OS	C T T P A Q G N S M F P S C C C T K P T D G N C
OS Δ A^{128}	C T T P _ Q G N S M F P S C C C T K P T D G N C
OS Δ Q^{129}	C T T P A _ G N S M F P S C C C T K P T D G N C
OS[N^{131} → A]	C T T P A Q G **A** S M F P S C C C T K P T D G N C
OS[M^{133} → Ox]	C T T P A Q G N S **M**(O) F P S C C C T K P T D G N C
OS[P^{135} → A]	C T T P A Q G N S M F **A** S C C C T K P T D G N C
OS[S^{136} → A]	C T T P A Q G N S M F P **A** C C C T K P T D G N C
OS[K^{141} → Me]	C T T P A Q G N S M F P S C C C T **K**(Me) P T D G N C
OS[P^{142} → A]	C T T P A Q G N S M F P S C C C T K **A** T D G N C
OS[D^{144} → N]	C T T P A Q G N S M F P S C C C T K P T **N** G N C

Fig. 7

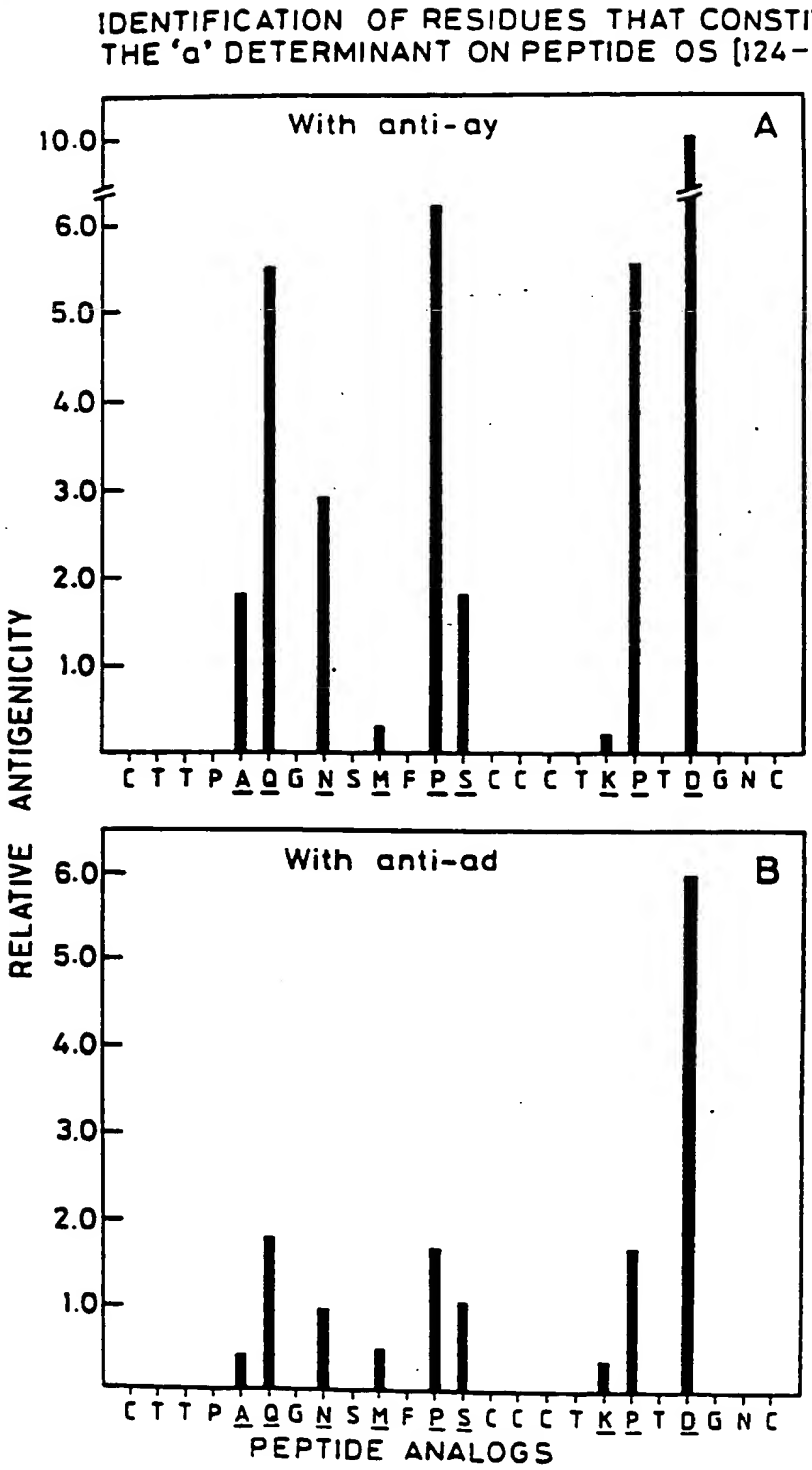
IDENTIFICATION OF RESIDUES THAT CONSTITUTE THE 'a' DETERMINANT ON PEPTIDE OS [124-147]
With anti-αγ
A
With anti-αδ
B
RELATIVE ANTIGENICITY
10.0
6.0
5.0
4.0
3.0
2.0
1.0
C T T P A Q G N S M F P S C C C T K P T D G N C
PEPTIDE ANALOGS

Fig.8

Reactivity of peptide OS[124-147] analogs with human anti-HBsAg serum

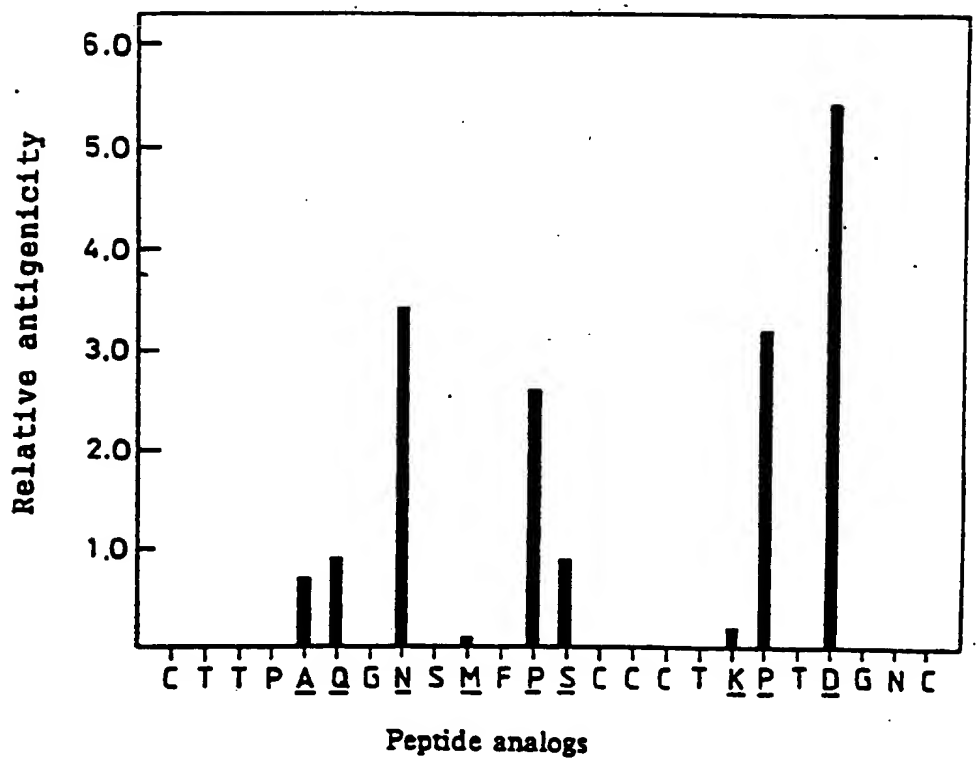

Fig. 9

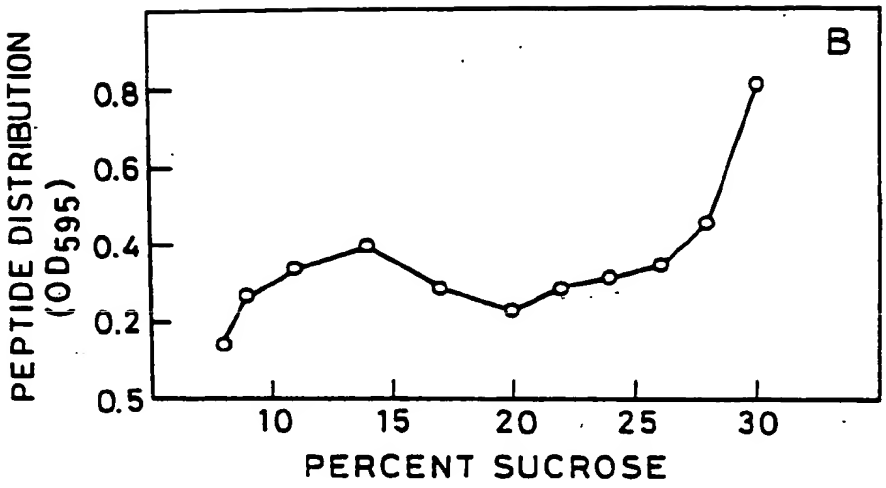

Fig. 10

Peptide	Subtype	Sequence
OS[D/N]	adw	CTTPAQGNSMFPSCCCTKPTNGNC
OS - ayr	ayr	CTIPAQGTSMYPSCCCTKPSDGNC

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/EP 93/02342

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/10 A61K39/29 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF IMMUNOLOGY. vol. 148, no. 12 , 15 June 1992 , WILIAMS AND WILKINS, BALTIMORE, US; pages 4006 - 4011 V. MANIVEL ET AL. 'A synthetic peptide spontaneously self-assembles to reconstruct a group-specific, conformational determinant of hepatitis B surface antigen'	1-4,7
Y	see page 4010, left column, line 30 - page 4011, left column, line 14	5

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

'A' document defining the general state of the art which is not considered to be of particular relevance
'E' earlier document but published on or after the international filing date
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'O' document referring to an oral disclosure, use, exhibition or other means
'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 1994	11 -02- 1994
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF IMMUNOLOGY. vol. 148, no. 12 , 15 June 1992 , WILIAMS AND WILKINS, BALTIMORE, US; pages 4012 - 4020 S.P. TRIPATHY ET AL. 'Design and synthesis of a self-assembling peptide derived from the envelope proteins of HIV type 1' see page 4019, right column, line 36 - line 42; figure 5	5
X	VACCINE vol. 10, no. 4 , April 1992 , BUTTERWORTH-HEINEMANN LTD., LONDON, GB; pages 204 - 208 K.V.S. RAO ET AL. 'Macromolecular self-association of a synthetic peptide derived from the hepatitis B surface antigen: construction of a quaternary epitope' see page 205, right column, line 29 - line 34; figure 3A	1-4,7
Y	see page 206, left column, line 12 - right column, line 2	5
P,X	JOURNAL OF IMMUNOLOGY. vol. 149, no. 6 , 15 September 1992 , WILIAMS AND WILKINS, BALTIMORE, US; pages 2082 - 2088 V. MANIVEL ET AL. 'Identification of a new group-specific determinant on hepatitis B surface antigen with a synthetic peptide' see page 2083, right column, line 1 - page 2087, left column, line 7	1-5,7
P,X	CLIN. EXP. IMMUNOL. vol. 90, no. 2 , 26 October 1992 , BLACKWELL, OXFORD,GB; pages 194 - 198 A. MISHRA ET AL. 'Immune response to hepatitis B virus surface antigen peptides during HBV infection' see page 197, right column, line 10 - line 13	1-4
P,X	VACCINE vol. 10, no. 12 , December 1992 , BUTTERWORTH-HEINEMANN LTD., LONDON, GB; pages 814 - 816 A. KUMAR ET AL. 'Comparison of immune responses to a native viral antigen and a synthetic peptide derived from it: implications for vaccine formation' see page 814, line 1 - line 12	1-4,6

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International Application No
PCT/EP 93/02342

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	VACCINE vol. 11, no. 3 , February 1993 , BUTTERWORTH-HEINEMANN LTD., LONDON, GB; pages 366 - 371 V. MANIVEL ET AL. 'A self-associating hepatitis B surface antigen-derived peptide that is immunogenic in alum' see page 366, line 1 - line 10	1-7
P,X	IMMUNOLOGY vol. 79, no. 3 , July 1993 , BLACKWELL SCIENTIFIC PUBLICATIONS,OXFORD, GB; pages 362 - 367 A. MISHRA ET AL. 'Human T-helper cell responses to a synthetic peptide derived from the hepatitis B surface antigen' see page 362, line 1 - line 12	1-4,6,7